



Molecular composition of raw peat and humic substances from permafrost peat soils of European Northeast Russia as climate change markers

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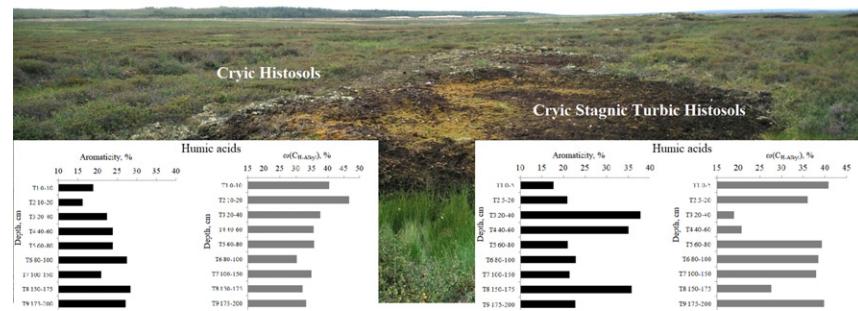
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HIGHLIGHTS

GRAPHICAL ABSTRACT

- Molecular composition of raw peats and HSs from permafrost peat soils studied.
- HAs and FAs were represented by a low amount of aromatic structures.
- HS structure was determined by intensity of cryogenic processes in the active layer.
- AR/AL of HA fragments can be used as markers of current climatic change.



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ABSTRACT

Humic substances (HSs) from the mire peat soils of the forest-tundra zone of the European northeast part of Russia have been characterized in terms of molecular composition. This was accomplished using solid-state ^{13}C nuclear magnetic resonance (^{13}C NMR) techniques and electron spin resonance (ESR) spectroscopy. The composition depended on the intensity of cryogenic processes in the active layer, the quality of the humification precursors (the degree of peat material transformation), and the biochemical selection of aromatic fragments during humification. Humic acids (HAs) and fulvic acids (FAs) of the peat soils showed the presence of compounds with a low extent of condensation and a low portion of aromatic fragments, which increased with depth. A higher proportion of aliphatic carbon species was found in the HAs, indicating a low degree of organic matter stabilization. Based on the data from the two types of peat soils, we suggest that particular changes in the proportion of aromatic and unoxidized aliphatic fragments on the border of the bottom of the active layer and permafrost layers can be used as markers of current climatic change.

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1. Introduction

Permafrost peatland ecosystems in the Arctic play an important role in global carbon sequestration, greenhouse gas emissions, and the regulation of the hydrological regime of tundra environments. They also

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provide stability to the biodiversity in the northern polar biome. The high-latitude peatland ecosystems of northern Russia are considered vulnerable to various climatic changes. Thawing and degradation of the permafrost release carbon compounds that have been immobilized. Permafrost degradation in the peatland ecosystems affects global and regional carbon balances (Zimov et al., 2006; Schuur et al., 2008).

The current degree of organic matter (OM) stabilization in permafrost-affected peatlands is considered to be underestimated. The functional role of humic substances (HSs) is manifested directly in soil stability, particularly in the formation of stable storages of carbon containing compounds, which are relatively resistant to degradation (Pefferkorn, 1997). The HS composition of peatlands has been revealed to have indexes related to various climatic stages (Swindles et al., 2012). This is expressed in botanical and palynological material composition in various Holocene paleoenvironments (Chambers et al., 2007). Differences in the composition of plant remnants also regulate the structural and functional parameters of HSs in the stratified layers of peat materials.

Nuclear magnetic resonance (NMR) spectroscopy is a valuable tool for the characterization of soil OM (SOM) and humification processes in soils. A major advantage is that it is a non-invasive method for solid soil samples or soil fractions (Kögel-Knabner, 2008). The content of functional groups and molecular fragments evaluated from ^{13}C NMR spectroscopy depends on the botanical composition of the peat and the degree of alteration that occurs to the organic remnant during decomposition.

The ratio of aromatic to aliphatic components is related to the type of peat material and content of oxygen-containing carboxylic and phenolic groups. This ratio also reflects the degree of humification (Zaccone et al., 2007; Klavinský and Purmalíš, 2013). Conditions of the permafrost affect soil formation, and together with low microbiological activity in forest-tundra, they lead to cryogenic denaturation and the transformation of highly dispersed humic acids (HAs). Thawing-freezing cycles result in intensive changes in the composition of humus and its colloidal physicochemical properties. These changes are manifested in increased HA mobility because of increased polydispersity of the molecules (Dergacheva and Dedkov, 1977). Thus, short- and long-term climatic changes could affect the quality of HAs. The alteration should be most pronounced in the supra-permafrost layers.

Numerous studies have substantiated the role of free radicals in biochemical reactions during humification. For example, semiquinone free radicals take part in humification (Kononova, 1966; Dick et al., 2005; Bayer et al., 2006). According to modern concepts, they play an important role in the step-by-step oxidizing polymerization process of humification. Free radical activity is also considered a fundamental property of HSs (Manhaes et al., 2002; Ariese et al., 2004). The free radical concentration is closely related to zonal environmental conditions and the anthropogenic impact of SOM (Jezierski et al., 2000; Lodygin and Beznosikov, 2010; Chukov et al., 2017).

Climate change and further risks related to intensive exploration of the Arctic sector of Russia are resulting in alterations to the hydrology of Arctic permafrost peat soils and irreversible changes in the peat layers. This results in degradation and thawing of the permafrost and uncontrolled greenhouse gas emission. Assessment of the transformations is therefore needed to estimate the role of peat soils in the global and regional stabilization of this organic material.

The aim of this work is to evaluate the degree of stabilization of SOM in permafrost peat soils based on the application of highly accurate instrumental methods: solid-state ^{13}C NMR and electron spin resonance (ESR) spectroscopy. The specific objectives were to (I) investigate the molecular composition and carbon species distribution in HAs and fulvic acids (FAs) of permafrost peat soils in relation to Holocene climatic parameters and (II) to evaluate the degree of molecule stabilization and biochemical activity based on free radical content.

2. Field sampling

2.1. Geographical setting

The peatlands investigated are in the north forest-tundra subzone (the basin of the Seida River, a part of the Pechora River basin), Vorkuta district, Komi Republic, Russia (Fig. 1). The permafrost in this region is extensive. The climate is cold, subarctic, and moderately continental (Kaverin et al., 2016). The annual mean air temperature is -4.0°C , the average sum of positive temperature values varies from 1150 to 1380°C , and the annual precipitation is ca. 550–600 mm. The evaporative power of the territory is 250 mm (Atlas on Climate and Hydrology of the Komi Republic, 1997).

2.2. Sampling sites

The following soils from the peatland complex were selected: soil from raised bog peats (Cryic Histosol) and soil from bare spots (Cryic Stagnic Turbic Histosol). The two peat soils differ in their active layer thickness and thermal regime. Sampling was conducted to a depth of 2.0 m. In the summer season, the permafrost table is fixed at a depth of ca. 40–60 cm. The peat material of the studied soils was dark brown (Munseel) and well decomposed. The material on the peatland surface is not continuous and is altered by wind corrosion and the cryogenic turbic effect. The total area of altered bare spots is about 8% of the total areas of peatland polypedones. Data on soil genesis and the main chemical characteristics have been published, as well as some data about soil physical chemistry and the botanical composition of the raw peat (Vasilevich and Beznosikov, 2017).

Cryic Histosols are typical of permafrost peatland with vegetation cover. The plot in question ($67^{\circ}03' \text{N}, 62^{\circ}56' \text{E}$) is 7 km southwest of Seida Railway Station. The landform is moraine lowland, and the diameter of the hillock is ca. 12 m. The upper limit of the permafrost is at a depth of 40 cm. The vegetation cover is formed by *Polytrichum commune*, *Empetrum hermaphroditum*, *Vaccinium vitis-idaea*, *Rubus chamaemorus*, *Vaccinium uliginosum*, and *Betula nana* along the edges of the hillock. Cryic Stagnic Turbic Histosols are typical of permafrost peatland without vegetation cover. The diameter of the polypeden in question ($67^{\circ}03' \text{N}, 62^{\circ}56' \text{E}$) is ca. 5 m. The upper limit of the permafrost is at a depth of 60 cm. Cryogenic crack formation was evident for bare sites (Fig. 2).

2.3. Physical and chemical properties of peat soils

Data on the composition and physical and chemical properties of peat soils (Table 1) showed that the ash content was quite different between the upper and lower sublayers. The first had a low content (3.8–6.0%) for both plots, while the second (the permafrost horizon) contained more mineral material than organic material. The lowest pH (3.5–3.9) values are typical of topsoil material and indicate strong acidity that is typical for oligotrophic peat soils. The pH values also increase with depth, which corresponds to the increasing ash content. The lower horizons showed enrichment in the exchangeable fractions of Ca and Mg, providing higher pH values (up to 5.5 in the middle part of the strata) and increased content of Fe, Al, and Ca. This is related to increased ash content in the peat materials. The highest C/N ratios were registered in superficial layers, while the lowest occurred in permafrost horizons. This indicated the greatest degree of OM alteration with depth. The fractional and group compositions of the SOM show a prevalence of HAs vs. FAs. The mass proportion of the HAs was highest in the superficial layers (up to 27.6%) and decreased towards the permafrost layers (Table 1).

2.4. Botanical composition and radiocarbon dating of peat layers

The beginning of peat material accumulation is related to the early Atlantic period (Table 1). The plots vegetated by *Picea* and *Betula* with presence of *Equisetum*, *Carex cespitosa*, and *Menyanthes* were related

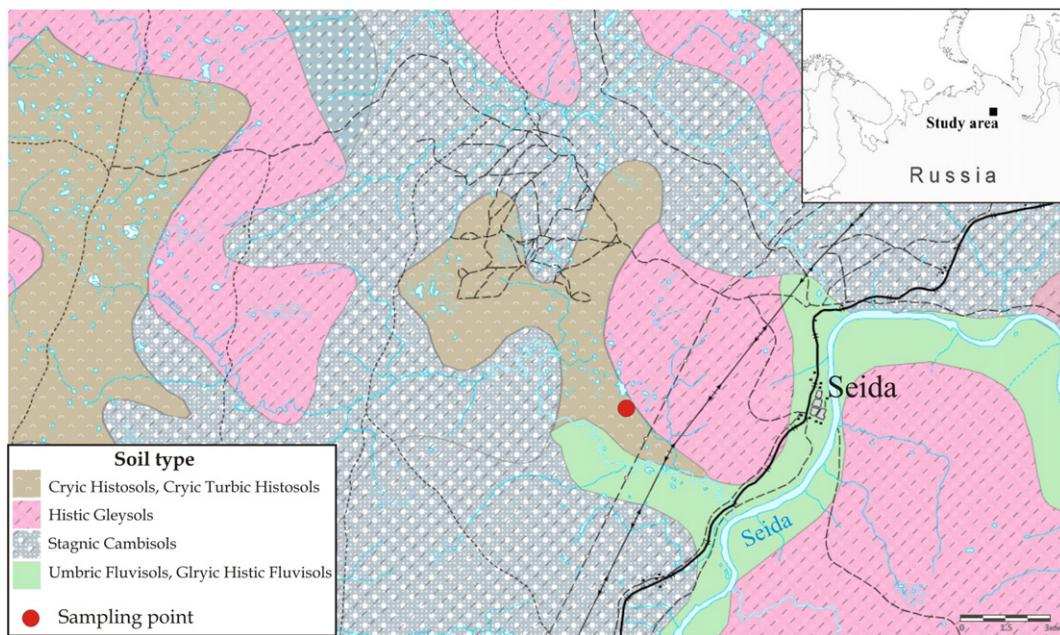


Fig. 1. Location of sampling area.

to the beginning of peatland formation. There was a sharp cooling and decrease in humidity in the middle Subboreal period and early Subatlantic period dated at 3754–1357 cal year BP (IGRAS 4641, 4640). This resulted in the appearance of permafrost layers (Andersson and Meyers, 2012; Routh et al., 2014), as well as the prevalence of mesotrophic (*Betula nana*, *Eriophorum*) and later oligotrophic communities of bryophytes, lichens, and bushes. The highest accumulation of peat is related to the Atlantic period (7984–6011 (5457) cal year BP; IGRAS 4646, 4642, 4648), when the linear rate of peat accumulation was 0.52–0.92 mm/year with a maximum in the Holocene optimum (7984–6473 cal year BP; IGRAS 4645, 4642).

With the beginning of the Subboreal at 6000 cal year BP, a substantial decrease in the accumulation of OM occurred according to the modified scheme of Blitt-Sernendes (Routh et al., 2014), which was applied to the northeastern part of Europe. This accumulation was due to humidification and decreasing temperature. The peat samples show a decrease in the remnants of woody species, with a dominance of *Betula nana*, *Salix*, and *Menyanthes*.

A sharp cooling and decrease in humidity occurred in the middle Subboreal period and early Subatlantic period at 3754–1357 cal year BP (IGRAS 4641, 4640), which began from the Middle Bronze Age cooling (near 3500 cal year BP). This resulted in the biomass increasing to 0.10 mm/year. Decreased intensity in plant growth and low phytocoenosis productivity resulted in a decrease in peat accumulation during the last 1500 years, which is estimated at 0.04 mm/year. There is

now no intensive accumulation of peat (Routh et al., 2014). Degradation of sphagnum cover and the appearance of indicator species that can be used as markers of various stages of peat soils development.

2.5. Temperature regimes of peat soils

The degree of SOM humification is mainly related to the temperature regimes of peat soils during the summer period (Orlov, 1995). Analysis of the temperature regimes of the studied soils showed that during the summer, soil with bare spots was heated and insulated more intensively than the soil with vegetated plots. This exemplified by the heating difference ($\Delta t > 0^\circ\text{C}$) being 2–5 times higher at depth > 10 cm than that of soils from vegetated plots. Soils from bare peat spots showed a trend of deeper cooling during the autumn-winter period (-1160°C days at a depth of 10 cm; -1099°C days at 20 cm). This is due to a thermal isolation effect of the moss layer in comparison with vegetated soils of the permafrost peat soils (-522°C days at a depth of 10 cm; -455°C days at 20 cm) (Vasilevich and Beznosikov, 2017).

3. Methods

3.1. Sample preparation

Samples from the active permafrost-free layers of peat soils were sampled from open pits with fixed-volume samples into the ground



Fig. 2. Study site.

Table 1

Properties of peat soils.

Depth, cm	Peat layer	Chronology of raw peat deposits		Peat type	Dominant botanical species	Degree of decomposition, %	Ash, %	pH H ₂ O	C/N	Gravimetric concentrations, %		
		¹⁴ C-age (year BP)	Calibrated age (cal year BP)									
		Median	1 σ range									
<i>Cryic Histosol</i>												
0–10	T1	1450 ± 70	1357	1295–1403	Raised	Polytrichum	20–25	5.4	3.75	30.1	16.6	10.9
10–20	T2	n.d. ^a	n.d.	n.d.	Raised	Subshrub	35–40	5.9	3.70	24.5	24.8	13.9
20–40	T3	3480 ± 70	3754	3644–3665	Fen	Carex, Eriophorum	30–40	4.2	3.74	21.4	21.8	13.9
40–60	T4	5230 ± 80	6011	5913–6026	Fen, frozen	Betula sp., wood	30–35	6.4	4.34	32.0	26.9	14.7
60–80	T5	5680 ± 80	6473	6352–6366	Fen, frozen	Menyanthes, Carex	30–35	33.1	4.86	22.4	11.2	6.8
80–100	T6	5710 ± 80	6508	6409–6567	Fen, frozen	Betula sp., wood, Carex	35–40	16.6	4.94	26.8	3.9	n.d.
100–150	T7	6130 ± 80	7022	6937–7158	Fen, frozen	Betula sp., wood, Carex	30–40	47.5	4.95	22.2	2.4	n.d.
150–175	T8	n.d.	n.d.	n.d.	Fen, frozen	Betula sp., wood, Carex	35–40	54.7	5.37	22.0	4.1	n.d.
175–200	T9	7160 ± 80	7984	7868–7898	Fen, frozen	Betula sp., Picea, wood, grass	50	86.3	5.33	17.2	3.4	n.d.
<i>Cryic Stagnic Turbic Histosol</i>												
0–5	T1	1310 ± 70	1231	1179–1298	Raised	Polytrichum	20–25	8.0	3.61	28.7	27.6	15.7
5–20	T2	n.d.	n.d.	n.d.	Transitional	Subshrub, Eriophorum	40–45	4.9	3.42	25.5	24.1	7.8
20–40	T3	4720 ± 70	5457	5326–5389	Fen	Carex	30–35	4.6	3.66	26.6	14.2	3.4
40–60	T4	5270 ± 80	6062	5942–5973	Fen	Hypnaceous, Carex	30–50	14.1	4.24	23.6	8.5	3.0
60–80	T5	n.d.	n.d.	n.d.	Fen, frozen	Subshrub, Carex	35–40	54.3	5.05	25.0	3.8	3.5
80–100	T6	n.d.	n.d.	n.d.	Fen, frozen	Carex	35	44.9	5.00	24.1	3.4	2.8
100–150	T7	n.d.	n.d.	n.d.	Fen, frozen	Betula sp., Picea, wood, Carex	35–40	65.1	5.47	23.4	5.0	2.6
150–175	T8	n.d.	n.d.	n.d.	Fen, frozen	Betula sp., Picea, wood, Carex	40–45	76.9	5.32	21.0	1.9	1.8
175–200	T9	n.d.	n.d.	n.d.	Fen, frozen	Equisetum, Betula sp., Picea, wood, Carex	>50	88.2	5.51	18.6	4.3	n.d.

^a n.d. – not determined.

with 10-cm increments. Samples from permafrost layer of peat soils were cored using steel pipes that were drilled into the ground in 20-cm increments, and intact frozen cores were retrieved between the drillings. Air-dried peat samples were homogenized and sieved through a 1-mm sieve. The powders of HAs and FAs were extracted from the peat material samples by double-extractions with 0.1 mol/dm³ NaOH according to the IHSS recommendations (Swift, 1996). The HAs were desalinated by dialysis. The FAs were purified on activated carbon AG-3 according to Forsyth and desalinated on cationic KU-2 (H⁺ form). The powders of HAs and FAs were obtained by heating at 35 °C in the baker. The concentrations of lipids in peat samples (100 g) were estimated by extraction with alcohol and benzene (500 cm³) at an approximate ratio of 1:2 in a Soxhlet apparatus for 12 h. Next, a major part of the solvent was removed using a rotary evaporator, and the residue was air dried (25 °C) (Lodygin and Beznosikov, 2005).

3.2. ¹³C NMR measurements

Measurements were carried out using a Bruker Avance III WB 400 NMR spectrometer (100.64 MHz for ¹³C). Solid-phase samples were placed in a 4-mm zirconium oxide rotor and spun at a frequency of 12.5 kHz at the magic angle. The cross-polarization sequence of excitation pulses was used for the registration of ¹³C spectra (CP/MAS). The contact time was 2 ms, the delay time was 1 s, and the number of scans was 50,000. Chemical shifts were referenced to low field signal of adamantane at 38.48 ppm.

3.3. ESR measurements

The ESR spectra were recorded using JES FA 300 spectrometer (JEOL, Japan) in an X-diapason with a free-radical modulation amplitude of 0.06 mT and a microwave power of 1 mW in the cavity. Magnesium powder with a fixed radical concentration was used as an external standard. The concentration of paramagnetic centers in powders was determined by comparison between the relative signal intensities of the external standard and the measured sample using the software JES-FA swEPR v. 3.0.0.1 (JEOL, Japan).

3.4. Temperature regimes

The temperature regime measurements were conducted using HOBO U-12 digital temperature loggers inserted into layers at depths of 0, 10, 20, and 50 cm. Temperature measurements were carried out once per hour.

3.5. Botanical composition

The botanical component compositions of the raw peats were determined in the Peatlands Ecosystems Laboratory of the Institute of Biology of Karelia Scientific Center of the Russian Academy of Sciences. Plant macrofossils were analyzed after deflocculating samples with a known volume (5–20 cm³) using 5% KOH and sieving (150-μm mesh) to remove fine detritus. The remains were identified under a stereo binocular (25–40 × magnification) based on reference literature. Sphagnum species were identified by their leaf morphology under microscope (100–400 × magnification).

3.6. Radiocarbon dating

¹⁴C dates of the peat layers were measured at the Institute of Geography of the Russian Academy of Sciences (lab code IG RAS). Radiocarbon activity was measured on an ultra-low-background liquid scintillation alpha/beta spectrometer (Quantulus 1220; PerkinElmer, Finland). The error of the dating method does not exceed 70–80 years for each date (Shishlina et al., 2014). The 1 σ range was calibrated using the radiocarbon calibration software Calib rev 7.1.0 and expressed in (median) calendar years before present (cal year BP; BP = 1950 CE).

4. Results and discussion

4.1. Structural molecular composition of the raw peats and HSs (¹³C NMR)

Analyses of the ¹³C NMR spectra (Fig. 3) of the majority of HSs and the raw peat samples revealed that the most expressed intensity of the signals in diapason at 0–47 ppm, which is related to aliphatic carbon. The high portion of aliphatic carbon is related to the accumulation of

organic precursors derived from organic remnants, such as long aliphatic chains from wax resins, fatty acids, cutin, and cell-wall phospholipids (Winkler et al., 2005; Jager et al., 2016). According to Hatcher et al. (1981), methyl groups related to the chemical shifts at 15–18 ppm are attributed to aliphatic fragments with developed spatial structures. Methylene atoms of carbon from long chains of hydrocarbons are related to the shifts at 29–33 ppm (Simpson and Simpson, 2009; Almendros et al., 2008).

The shift area of the short aliphatic chains is related to the shifts at 35–47 ppm (Wilson, 1981).

The region at 47–60 ppm with a maximum at 56 ppm is related to the methoxy groups of lignin fragments (syringyl and guaiacyl propane units) (Kovaleva and Kovalev, 2015; Tadini et al., 2015). In this area of the spectra, α - and β -carbon atoms of amino acids show an evident reflex (Wilson, 1981; Keeler et al., 2006; Knicker et al., 2008). The most

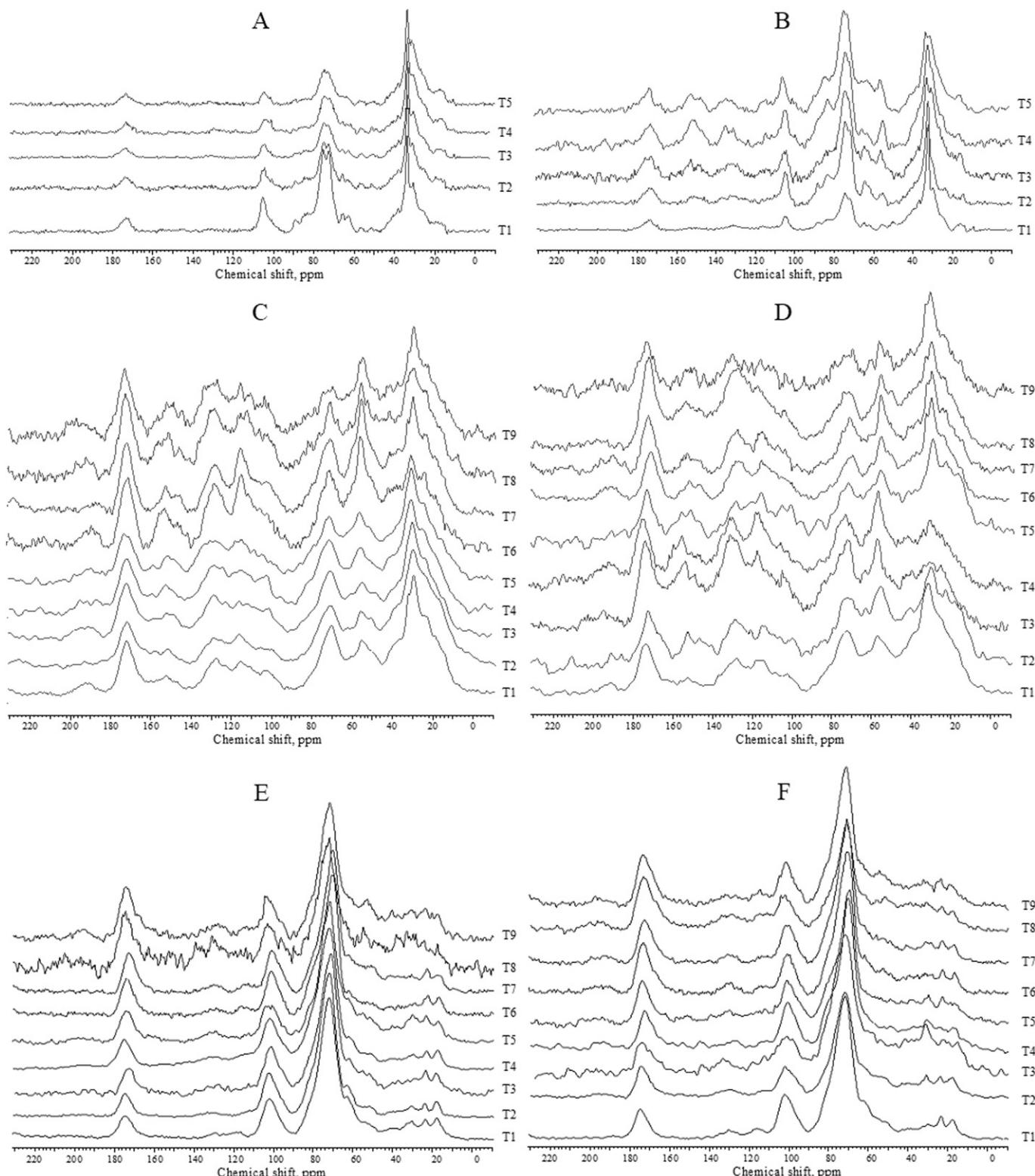


Fig. 3. CPMAS ^{13}C NMR spectra of raw peats (A, B), HAs (C, D) and FAs (E, F) isolated from Cryic Histosol (left) and Cryic Stagnic Turbic Histosol (right).

intensive peak in the region of 60–108 ppm with a maximum at 70–71 ppm in the ^{13}C NMR spectra is related to $\text{CH}(\text{O})$ groups from the ring atoms of cellulose and other carbohydrate fragments (Keeler et al., 2006; Simpson and Simpson, 2009). In most of the HA powders, the signals at 62 ppm were fixed and are possibly related to CH_2O groups from the hexoses of polysaccharide fragments (Duarte et al., 2008). This peak is more pronounced in the FAs. Also, the FAs showed an intense peak at 101 ppm due to anomeric acetalic atoms of carbon and structural fragments of polysaccharides (Zech et al., 1992).

In the region of 108–144 ppm, unsubstituted or alkyl-substituted aromatic carbon atoms are expressed, while in the region of 144–164 ppm, their analogues containing groups -OH, -NH₂, and -OCH₃ are expressed. The peaks at 128 and 137 ppm from aromatic rings are connected with the main parts of molecules by aliphatic chains. The signal at 147–149 ppm is typical for structural blocks of lignin and for oxygen compensated atoms of aromatic rings (syringyl and guaiacyl propane units) (Keeler et al., 2006; Almendros et al., 2008; Kovaleva and Kovalev, 2015). The spectra have higher intensity in this range for HAs than for FAs. In the range of 164–183 ppm with a maximum at 172–174 ppm, fixed atoms of carboxyl fragments, polypeptides, and carbonyl-amides occur (Keeler et al., 2006; Lodygin et al., 2014). The atoms of quinone fragments and the carbonyl groups of ketones and aldehydes provide weak signals in the ranges of 183–190 and 190–204 ppm, respectively. Detailed interpretations of the ^{13}C NMR spectra are given in Tables 2 and 3.

^{13}C NMR analyses of the raw peat samples from the two peat soils investigated demonstrate a substantial contribution of aliphatic fragments in the structure of the molecules. The portion of paraffin structures is as high as 62%, and total portion of carbohydrate fragments is up to 52% in different raw peats (Table 2). These components prevail in peat-forming organic precursors (bryophytes and shrubs) (Klavinš and Purmalis, 2013).

The spectroscopy of a specific peak at 29–33 ppm from long aliphatic chains indicates the accumulation of unsaturated and saturated fatty acids (natural resin), complex esters (waxes), and *n*-alkanes from the plants in the northern conditions (Lehtonen et al., 2001). The waxes are considered as a part of the cuticula of many plants, especially plants that are inhabitants of soils with low oxygen availability (Andersson and Meyers, 2012). ^{13}C NMR data from the raw peats indicate a substantial portion of paraffin fragments (up to 61%; Table 2). The content of lipid fractions in the peat materials was highest in the superficial horizons (up to 15.7%; Table 1). This is attributed to the increased portion of paraffin structures in the polytrichum and bush types of raw peats. This corresponds well with previously published data (Gondar et al.,

2005; Klavinš and Purmalis, 2013). It was shown that humification leads to a decrease of the lipid fractions by as much as 10 times (Savel'eva et al., 2010). It has also been revealed that the humification process results from the partial transformation of linear structures with the appearance of more sterically branched molecular ensembles of paraffin fragments. These processes are directly connected with the biological activity of the peat soils and are most expressed in soil from the bare spots.

The aromaticity of the peat material from bare spots is 1.5–5.0 times higher on the border of the solum with the permafrost table than in soils from vegetated peat areas. The oxidized lignin-derived compounds of aromatic structures and methoxy groups prevail in soils from unvegetated polypedones.

The quantitative compositions of ^{13}C NMR spectra revealed a high portion of unoxidized aliphatic fragments in the HA molecules. The lowest intensities of the signal at 0–47 ppm was fixed for the HAs for Cryic Stagnic Turbic Histosol from the depths of 20–40 cm and 40–60 cm on the border solum with the permafrost table. The highest concentration was in the HAs from superficial layers. Lower in the soil profile, the contribution of branched aliphatic structures decreases, especially for the HAs extracted from the permafrost layers from bare spots (Fig. 3).

Quantitative analyses of the structural components of the HAs revealed a low portion of aromatic carbon in the HAs and FAs (15.2–36.3 and 1.6–12.8%, respectively). The content of functional groups was different in seasonally thawed layers and permafrost layers. Specifically, the aromatic fragments and methoxy groups show increasing trends and paraffin groups show decreasing trends with respect to depth (Table 3). This directly reflects the interrelationships between the structural composition of the HAs and climatic conditions of the Holocene period, and it could be related to periods of warm climate (Vasilevich and Beznosikov, 2017).

In the active layers of the unvegetated permafrost peat soils, the portion of aromatic fragments of HA showed a substantial increase with depth. Dynamic thawing and freezing on the border of the permafrost results in increased aromaticity and a decreased portion of unoxidized aliphatic fragments. This is most pronounced on the border of permafrost. The indexes correlated with the elemental composition data, which indicate a higher degree of condensation of the HA molecules (Vasilevich and Beznosikov, 2017).

Long periods of low temperatures lead to the destruction of the easily decomposable parts of molecules and to transformation of the main part of molecules. Nevertheless, soil from the unvegetated permafrost peatland areas are more intensively heated during the summer period. This results in more raw peat decomposition in seasonally thawed

Table 2

Percentage of carbon in the main structural fragments of raw peats (according to ^{13}C NMR data).

Horizons/depth, cm	Chemical shift, ppm								Integrated indicators	
	0–47		47–60		60–108		108–144		AR/AL ^a	fa, ^b %
	Alkyl	O,N-alkyl	Aromatic	Carboxyl, ester, amide	Quinone	Carbonyl				
Cryic Histosol										
T1 0–10	36.8	0.9	55.5	1.7	0.7	4.2	0.0	0.1	0.025	2.43
T2 10–20	47.6	1.1	44.4	1.2	0.7	5.0	0.1	0.0	0.020	1.95
T3 20–40	54.1	1.5	37.4	0.9	0.2	5.4	0.2	0.2	0.013	1.30
T4 40–60	57.4	1.8	34.8	1.6	0.2	3.7	0.1	0.3	0.019	1.88
T5 60–80	61.3	1.3	30.5	1.5	0.8	4.6	0.0	0.2	0.024	2.32
Cryic Stagnic Turbic Histosol										
T1 0–5	50.6	4.1	33.2	5.8	1.2	4.9	0.0	0.2	0.076	7.05
T2 5–20	33.1	2.1	49.5	6.6	2.6	5.3	0.2	0.5	0.104	9.42
T3 20–40	40.1	4.7	40.1	5.6	4.0	5.2	0.0	0.2	0.107	9.63
T4 40–60	32.9	2.5	45.2	7.0	7.1	4.7	0.3	0.3	0.167	14.35
T5 60–80	30.7	5.4	48.2	5.7	4.5	5.2	0.2	0.1	0.117	10.45

^a The signals from the aromatic structures (Ar) were summed for the regions of 108–164 and 183–190 ppm; those from the aliphatic structures (AL) were summed for the regions of 0–108, 164–183, and 190–204 ppm.

^b Aromaticity.

Table 3Percentage of carbon in the main structural fragments of HAs and FAs (according to ^{13}C NMR).

Horizon/depth, cm	Chemical shift, ppm								Integrated indicators	
	0–47 Alkyl	47–60 O,N-Alkyl	60–108	108–144 Aromatic	144–164	164–183 Carboxyl, ester, amide	183–190 Quinone	190–204 Carbonyl	AR/AL ^a	fa, ^b %
Cryic Histosol										
Humic acids										
T1 0–10	40.4	10.4	22.0	13.6	3.9	8.4	0.3	1.0	0.217	17.81
T2 10–20	46.6	10.5	20.3	11.2	3.8	7.4	0.2	0.1	0.180	15.22
T3 20–40	37.6	10.2	22.2	15.4	5.3	8.3	0.3	0.7	0.266	21.03
T4 40–60	35.5	10.4	23.2	16.9	4.9	7.7	0.7	0.7	0.290	22.49
T5 60–80	35.6	11.4	22.1	16.9	4.8	7.5	0.9	0.9	0.291	22.56
T6 80–100	30.4	14.5	20.4	19.4	5.3	7.8	1.1	1.0	0.349	25.85
T7 100–150	34.8	15.8	21.5	16.0	3.1	8.0	0.5	0.2	0.244	19.61
T8 150–175	32.1	11.9	19.9	20.7	5.9	8.8	0.2	0.6	0.365	26.71
T9 175–200	33.2	12.2	18.5	20.6	5.1	9.1	0.1	1.4	0.347	25.75
Fulvic acids										
T1 0–10	12.1	6.6	74.0	1.6	0.0	5.7	0.0	0.0	0.016	1.62
T2 10–20	7.1	4.5	80.1	2.3	0.1	5.7	0.0	0.1	0.024	2.34
T3 20–40	11.7	7.2	72.4	3.0	0.1	5.5	0.0	0.1	0.032	3.08
T4 40–60	9.8	5.0	64.7	10.2	2.2	6.7	0.5	1.0	0.147	12.83
T5 60–80	16.2	9.0	61.2	4.5	1.0	7.3	0.1	0.6	0.059	5.60
T6 80–100	10.7	5.0	70.8	3.4	0.2	9.6	0.0	0.2	0.038	3.63
T7 100–150	15.3	10.0	61.9	3.1	0.5	9.1	0.0	0.1	0.038	3.62
T8 150–175	14.5	7.7	55.0	9.2	2.2	11.1	0.0	0.2	0.129	11.42
T9 175–200	16.9	10.1	56.4	3.7	0.8	11.5	0.0	0.5	0.047	4.52
Cryic Stagnic Turbic Histosol										
Humic acids										
T1 0–5	40.8	10.9	22.2	14.0	2.9	8.5	0.1	0.5	0.205	16.98
T2 5–20	36.1	12.6	24.0	14.9	4.7	7.5	0.1	0.2	0.246	19.71
T3 20–40	19.0	8.9	24.6	25.1	10.8	9.7	0.4	1.5	0.571	36.34
T4 40–60	20.7	12.6	23.5	25.1	7.5	8.7	0.9	1.1	0.502	33.44
T5 60–80	39.3	12.2	22.8	14.2	5.0	6.1	0.2	0.1	0.242	19.47
T6 80–100	38.4	12.6	18.0	17.0	3.5	8.0	0.9	1.6	0.272	21.39
T7 100–150	38.0	13.4	18.8	16.1	2.7	8.3	1.3	1.5	0.251	20.07
T8 150–175	27.7	9.4	17.2	25.3	9.2	10.8	0.0	0.4	0.526	34.46
T9 175–200	39.8	10.2	16.7	17.3	4.8	10.0	0.3	0.9	0.289	22.39
Fulvic acids										
T1 0–5	11.2	6.8	69.8	4.6	0.4	6.8	0.2	0.2	0.055	5.22
T2 5–20	17.2	8.6	59.0	4.6	1.1	8.7	0.1	0.7	0.061	5.78
T3 20–40	24.2	8.9	54.0	5.8	0.7	6.1	0.0	0.2	0.070	6.54
T4 40–60	18.2	7.9	61.1	2.5	1.3	8.1	0.0	0.9	0.040	3.82
T5 60–80	18.3	8.1	55.8	6.8	1.6	9.4	0.0	0.1	0.091	8.32
T6 80–100	15.0	7.9	57.9	3.4	1.2	12.4	0.5	1.8	0.053	5.07
T7 100–150	15.4	8.8	59.2	3.0	1.3	10.6	0.5	1.3	0.050	4.79
T8 150–175	17.9	9.8	47.4	8.4	1.4	14.3	0.0	0.8	0.108	9.75
T9 175–200	14.9	9.4	59.3	3.6	0.4	12.1	0.0	0.3	0.041	3.98

^a The signals from the aromatic structures (Ar) were summed for the regions of 108–164 and 183–190 ppm; those from the aliphatic structures (AL) were summed for the regions of 0–108, 164–183, and 190–204 ppm.

^b Aromaticity.

layers. The index increases from 20–25% to 40–50% for superficial layers and layers bordering the permafrost, respectively. An increase of up to 55% was found in the portion of sedge remnants in the composition of peat-forming remnants. Soils formed under stable vegetation cover show lower degrees of peat decomposition: 20–25% in superficial layers and 30–35% in permafrost border layers. The deepest humification is precisely related to these indexes in soil from bare spots (Routh et al., 2014).

It should be emphasized that there are substantial structural differences in the HAs of the two soil types. Field data from Savel'eva et al. (2010) indicate that the humification process is more active in sedge-type peats. These processes initiate in tissues of the sedges and can be established in the increase in signals from aromatic carbon species in the ^{13}C NMR spectra. This means that the active layer of the peat soils is present by the modern stage of OM transformation, and this process is more intensively pronounced in soil from bare spots. The higher indexes of the positive temperatures in soils from bare spots simulate the climate change and result in transformation of the structural parameters of humic acids. These transformations can be regarded as an intensification of diagenesis of the peat sediments. This involves

carbonification accompanied by processes of dehydration and demethylation of HSs molecules, resulting in reductions of unoxidized paraffin fragments and probably resulting in the release of methane. In this regard, it is important to note that the formation of unvegetated peat spots is a modern process, which has probably occurred no earlier than 300–400 years ago (Routh et al., 2014).

The low differentiation of humic acids composition in peat layers under the permafrost table is caused by many periods of restratification of the peat materials on the border of the early and middle Holocene. Partial isolation of the peat materials determines the degree of peat destruction. The lower horizons were partially buried and isolated with less pronounced intensity of the soil formation, which almost stopped with the formation of the permafrost layers in the Subboreal period (Chimitdorjieva, 2016). For the relic peat sampled from depths of 150–175 cm, intense maximums of aromatic carbon were revealed for both HAs and FAs (Table 3). These peaks are related to the Holocene climatic optimum (Roland et al., 2015), which is dated at about 7000 cal BP (IG RAS 4645, 4646).

The content of the labile forms of humus is related to weak biological activity and reductive conditions in the peat layers. Partially isolated OM

shows very slow transformation trends. The portion of labile carbohydrate fragments of the HAs decreases slowly with the soil depth. Similar data were obtained for peatlands in Latvia (Klavins and Purmališ, 2013). It is well known that bacteria play a more active part in the humification of OM of peat materials in comparison with micro fungi, saccharomyces, and actinomycetes (Chimitdorjjeva, 2016).

FAs are more enriched by oxygen-containing fragments but have fewer of the paraffin structures (up to 24.2%). The similar content of carboxylic groups in HAs and FAs revealed in peat soils is unusual for mineral soils (Lodygin et al., 2014). The carboxylation process leads to an increase of –COOH groups with the soil depth and to a higher portion in the FAs of the lower layers. The high portion of carbohydrate fragments in FAs (up to 80.1%) results in increased bioavailability of the FA molecules in comparison with the HAs (up to 24.6%).

The main difference between FAs and HAs is expressed in the content of methoxy groups, amino acid fragments (shifts between 47 and 60 ppm), and aromatic and aliphatic carbonyl groups. The lower portion of groups associated with the shift at 47–60 ppm (up to 10.1% in FAs and 15.8% in HAs) is related to the lower portion of nitrogen of amino groups (Vasilevich and Beznosikov, 2015). Alteration of the structural composition of the FAs can be considered to be the most flexible for the soil formation conditions. Differentiation of alkyl, methoxy, and amino groups results from changes in the soil temperature regimes and types of botanical composition of peats during the different Holocene periods. The highest content of aromatic fragments in the FAs of the T4 layer is probably connected with sharp freezing-thawing cycles.

4.2. Statistical analysis

The representative data array obtained after statistical processing allowed us to analyze the main trends of humification. Correlation analysis was conducted using the Pearson criteria (normal distribution). One-way ANOVA was used to evaluate the influence of factors (aromaticity, content of unoxidized fragments, and spin-electrone values) on the structural-functional composition of humic substances in various layers of the peat materials. The results were considered as significant in cases of p values < 0.05 .

Climatic features determine not only the qualitative composition of the main peat-forming plants, but also the degree of biopolymer degradation, including depolymerization of polyphenolic components of lignin structures, decomposition of proteins, and polysaccharides through the action of bacteria and fungi. Lignin components are considered to largely define the degree of HS condensation. Comparing the data on the botanical composition of various plants shows that the lignin content in lichens and mosses (8–10%) is lower than in sedge (15–20%), shrub, and woody (20–30%) plant materials (Kovaleva and Kovalev, 2009). Based on this data, the portion of lignin in organic remnants was calculated while taking into account the botanical composition of raw peats.

Methoxyl groups are associated with mostly phenilpropanous fragments of lignin-type aromatic components. This was confirmed by the correlation of the methoxy group content with the portion of oxidized aromatic fragments in HAs and FAs ($n = 36$; $r = 0.50$, $r_{cr} = 0.33$, $p = 0.95$). The indexes of aromaticity and the portion of carboxylic groups of HAs are also correlated ($n = 18$; $r = 0.64$, $r_{cr} = 0.47$, $p = 0.95$). This indicates the main trends of HA development during the humification process (carboxylation and saving the most stable groups). A significant negative correlation of unoxidized aromatic and aliphatic fragments was revealed ($n = 18$; $r = -0.94$, $r_{cr} = 0.47$, $p = 0.95$). The results of one-way ANOVA ($F = 15.77$, $p = 0.0014$) cannot be explained by only the degradation of parapheninous structures as stochastic processes. This could be the result of the cyclization of aliphatic chains with subsequent dehydrogenation and the formation of aromatic fragments.

The degree of peat decomposition positively correlates with the methoxy groups ($r = 0.47$) and carboxylic ($r = 0.61$) groups of

FAs ($n = 18$, $r_{cr} = 0.47$, $p = 0.95$). There was also a negative correlation of this parameter with the portion of carbohydrate fragments of the HAs ($r = -0.56$). The calculated lignin content correlates with the portion of oxidized aromatic fragments ($r = 0.49$) and carboxylic group content in FAs ($r = 0.52$). The degree of condensation of HA molecules is not an index of peat decomposition in all cases and is related to its botanical composition. The structure of the aromatic fragments of HAs is related to the portion of sedge remnants ($r = 0.53$, $r_{cr} = 0.47$, $p = 0.95$), while the portion of paraffin fragments correlates with the composition of subshrubs ($r = 0.53$). The structure of aromatic fragments of FAs is related to the portion of wood remnants ($r = 0.57$), while carbohydrates show a correlation with the composition of subshrubs ($r = 0.72$) and mosses ($r = 0.52$). The portion of unoxidized aromatic fragments of HAs also demonstrates a correlation with the occurrence of plants such as *Carex aquatilis* ($r = 0.54$), *Carex cespitosa* ($r = 0.47$), and *Menyanthes* ($r = 0.55$) in the peat material. The occurrence of *Carex rostrata* ($r = 0.63$) and *Carex cespitosa* ($r = 0.47$) correlates with the portion of methoxy groups of HAs ($r_{cr} = 0.47$, $p = 0.95$).

4.3. ESR of the raw peat and humic acids

Analyses of the ESR spectra of the raw peat samples and HAs fixed in the interval of free organic radicals shows that all the spectra presented an intense singlet line with a *g*-factor close to 2. This indicates the presence of free radicals localized on aromatic structures. The presence of a signal with Loren's form indicates that the spin-spin contacts in the molecules are substantial (Senesi, 1990; Scott et al., 1998). The integral intensity of the signals allows us to assess the concentrations of the paramagnetic centers of free radicals in the HA powders. The concentration of paramagnetic centers is higher in the HAs than in raw peats, which is strongly related to higher values of aromatic and other polycondensed structures in the HA molecules than in peat materials (Table 4).

A correlation was revealed between concentrations of free radicals in the HAs and raw peat samples ($r = 0.65$, $n = 10$, $p = 0.95$, $r_{cr} = 0.63$), which indicates the localization of paramagnetic centers of peat organic matters mainly in the HAs. The content of free radicals in raw peats and HAs shows decreasing trends with respect to depth down to about 60 cm, and in the layer 60–80 cm only in peat soil without vegetation some increasing of this parameter (Table 4). Higher paramagnetic activity of HAs is evident in the layers 80–150 cm, which is caused by

Table 4
Free radicals concentration in peat soils and HAs.

Horizons	Depth, cm	Free radical concentration, 10^{15} spin/g	
		Raw peat	Humic acids
<i>Cryic Histosol</i>			
T1	0–10	1.54	3.76
T2	10–20	0.64	2.41
T3	20–40	0.55	1.88
T4	40–60	0.40	1.32
T5	60–80	0.31	1.34
T6	80–100	n.d. ^a	5.79
T7	100–150	n.d.	6.03
T8	150–175	n.d.	1.97
T9	175–200	n.d.	1.18
<i>Cryic Stagnic Turbic Histosol</i>			
T1	0–5	0.57	1.65
T2	5–20	0.30	0.72
T3	20–40	0.39	0.50
T4	40–60	0.46	0.31
T5	60–80	1.37	1.20
T6	80–100	n.d.	3.41
T7	100–150	n.d.	3.76
T8	150–175	n.d.	0.31
T9	175–200	n.d.	1.01

^a n.d. – not determined.

changes in the botanical composition of the raw peats and a higher portion of wood and sedge remnants containing syringil fragments from lignin-derived compounds. The content of paramagnetic centers of HAs from Cryic Histosol is higher than in HAs from Cryic Stagnic Turbic Histosol, due to the different contents of methoxy and quinone groups. This is also supported by the strong correlation between the concentration of paramagnetic centers and the mass portion of methoxy groups, as well as the correlation between quinone groups ($r = 0.69$ and $r = 0.45$ respectively, $n = 18$, $P = 0.95$, $r_{cr} = 0.47$). As shown previously, the sedge peats have increased paramagnetic activity (Sartakov et al., 2015).

5. Conclusions

For the first time, this study identified the molecular composition of raw peats and humic substances isolated from the permafrost peat soils of the Bol'shezemel'skaya Tundra in European North-East Russia. It was shown that climatic factors are the main driver of the formation of SOM as well as the quality and degree of natural polymer destruction. These parameters are reflected in the functional groups and molecular compositions of the humic substances studied in both relic and modern formed peat layers.

HAs and FAs of the permafrost peat soils presented low-density molecular structures with a low portion of carbon species from aromatic fragments (15.2–36.3% for HAs and 1.6–12.8 for FAs). In comparison with the mineral soils, the content of carboxylic groups was lower in the peat materials. This reflected the anaerobic conditions in the permafrost peat soils. The content of functional groups and molecular fragments in the HAs illustrated a trend of decreasing paraffin groups and the accumulation of aromatic species of HAs, as well as carboxylic groups from FAs along the depth of the peat soils profile. This resulted from the higher degree of SOM transformation.

Transformation of the linear aliphatic fragments leads to the cyclization of paraffin chains with the formation of stable aromatic fragments. As a result of the low biochemical activity of the permafrost peat soils, labile fractions of carbohydrates demonstrate a low transformation rate. The effects of low temperatures result in increment of the aromatic fragments of HAs extracted from the layers in contact with the permafrost. The highest indexes of biological activity from bare permafrost peatlands indicate possible climate changes in cases of increased soil temperatures. This results in a stronger alteration of the SOM content in the solum active layer. We conclude that the data on SOM content and molecular quality can be used as an indicator of the degree of OM alteration in the sequence of vegetated and bare permafrost peat soils.

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